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The *FHY3* and *FAR1* genes encode transposase-related proteins involved in regulation of gene expression by the phytochrome A-signaling pathway

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Summary

The *Arabidopsis* mutants *far1* and *fhy3* display a phenotype of reduced inhibition of hypocotyl elongation, which is specific to far-red light and therefore specific to the phytochrome A (phyA)-signaling pathway. We report that the proteins encoded by the *FAR1* and *FHY3* genes are both related to the transposases of type II *MuDR* family transposons. We demonstrate that the FAR1 protein is capable of activating transcription in *Arabidopsis*, indicating that it may define a type of transcriptional regulator. Using microarray expression analysis, we show that of 293 mRNAs twofold induced in wild-type Col-0 plants by continuous far-red light, 85% show reduced responsiveness in the *fhy3* mutant. Notable alterations were observed in the responses of genes encoding certain transcription factors, proteins involved in cell wall extension, and proteins related to redox balance control. We also found genes, including some involved in transcriptional control, which showed altered transcriptional behavior in the dark-grown mutant plants. Taken together, our data suggest that FAR1 and FHY3 may function 'permissively' outside the signal transduction pathway of light-regulated development, yet be required for the expression of transcriptional regulatory components. An alternative possibility is that their role includes both light-signal transduction and transcriptional regulation of other genes not responsive to light. We propose that FAR1 and FHY3 control the expression of their target genes by a mechanism that has evolved directly from the way that an ancestral, MuDRA-like transposase bound to the TIRs of mobile elements.

Keywords: phytochrome, microarray, signaling mutant, transposase, MULE.

Introduction

The phytochrome systems are a part of a system of photoreceptors that confer sensitivity to ultra-violet, visible, and infrared radiation on higher plants. Five phytochromes exist in *Arabidopsis*, designated phyA–E. The phyA receptor is required for the response of etiolated seedlings to far-red light (FRc, centered on 730 nm) given alone (Quail, 1998). As etiolated plants lacking phyA are effectively blind to light of this wavelength, phyA is a useful model system for the genetic analysis of light signaling because all the signals are known to originate from a single receptor. A number of mutations are known that interfere with signal transfer from phyA to the machinery of photomorphogenic development. One class of mutants is hyposensitive to FRc. These include *fhy1, fhy3, far1, hfr1, fin219, pat1, pat3, laf1, laf6,* and *fin2* (Ballesteros *et al.*, 2001; Bolle *et al.*, 2000; Desnos *et al.*, 2001; Fairchild *et al.*, 2000; Hsieh *et al.*, 2000; Hudson *et al.*, 1999; Moller *et al.*, 2001; Soh *et al.*, 1998; Wang and Deng, 2002; Zeidler *et al.*, 2001) and are reviewed by Hudson (2000). *far1* and *fhy3* are closely related members of the *FAR1* gene family.

There are also two mutants, *spa1* (Hoecker *et al.*, 1999) and *eid1* (Buche *et al.*, 2000), that confer hypersensitivity to FRc and are thought to encode negative regulators of phyA signaling. These mutants were isolated by virtue of their shorter than usual hypocotyls in FRc. *SPA1* and *EID1* encode a WD-repeat protein and an F-box protein, respectively, and may both therefore be implicated in ubiquitin-mediated protein-degradative pathways.

A general conclusion has been drawn from the phenotypes of the above mutants. As they show a specific lesion

in FRc perception, they are likely to be specific to the phyA-signaling pathway (Fankhauser and Chory, 1997; Hudson, 2000). From this has followed the secondary conclusion that these mutant gene products are likely to act as early signaling components, i.e. they are signal transduction components downstream of phyA, but upstream of any confluence point between the phytochromes or other photoreceptors.

Transcriptional control in response to light may be closely tied to the primary signaling function of the phytochrome system. Phytochromes are capable of entering the nucleus, and they do so in response to light (Hisada *et al.*, 2000; Kircher *et al.*, 1999). The 'active' Pfr form of phytochromes A and B interacts with a nuclear basic helix-loop-helix transcription factor, PIF3 (Ni *et al.*, 1998, 1999), which, in turn, recognizes the known light-regulatory *cis*-element, G-box (Martinez-Garcia *et al.*, 2000). Microarray analysis has identified a downstream set of genes, which are rapidly induced by far-red light in wild-type *Arabidopsis* (Tepperman *et al.*, 2001). The induction of these genes is completely lost in a phyA photoreceptor-null mutant, indicating that the induction of transcription of these genes is attributable to a signal from the phyA photoreceptor. Significantly, a large number of these early responding genes encode transcription factors, making them excellent candidates for the first components in the transcriptional network that drives the photomorphogenic developmental program. The mutations that appear to specifically affect phyA signaling are therefore likely to affect transcriptional light responses. In this paper, the phenotypes of the *far1* and *fhy3* mutants are characterized at the molecular level, with the aim of identifying which genes are downstream of the signaling pathways or branches defined by these mutants. These data allow us to determine that the role of these signaling components is upstream of any transcriptional hierarchy in phyA signaling, and that FAR1 and FHY3 may act independently of the phyA-signaling pathway.

Results

The FAR1 and FHY3 proteins are closely related to each other and to Mutator-like element (MULE) transposases

All of the currently known phyA-signaling mutants were isolated in genetic screens for altered responses to FRc at the seedling stage. The most obvious response of seedlings to FRc is an inhibition of the elongation of the hypocotyl. Most of the known phyA-signaling mutants display varying degrees of altered hypocotyl elongation when grown in FRc (Hudson, 2000). The seedling phenotypes of the mutants and wild types used in this study, together with the null *phyA-101* and RLD lines used by Tepperman *et al.* (2001) are shown in Figure 1(a). Both *fhy3* and *far1* respond to FRc, whereas *phyA* is completely insensitive. Note that the response is stronger (i.e. closer to wild type) in *far1* than in *fhy3*.

The *FAR1* gene was originally described as a member of a gene family of unknown function (Hudson *et al.*, 1999). Since then the *Arabidopsis* genome has been completely sequenced (Arabidopsis Genome Initiative, 2000), revealing that the closest homolog of *FAR1* lies on the top of chromosome 3. This gene has now been defined as the *fhy3* locus (Wang and Deng, 2002). The gene family defined by *FAR1* has since been found to share homology with a number of other uncharacterized sequences in *Arabidopsis*, all of which share homology to the MULE family of transposons (Lisch *et al.*, 2001). We have noted that a more recently characterized transposon of maize, *Jittery* (GenBank Acc. # AF247646), contains an open reading frame showing yet more substantial peptide-level homology to the *FHY3* and *FAR1* genes than other *Mutator* family members. *Jittery* is described, by the submitting authors, as an active transposon of the *Mutator* type. Figure 1(b) shows the protein-level similarity between the pfam00872-defined transposase domain of the predicted proteins encoded by *Jittery* and *MuDR*. It also shows that *FAR1* and *FHY3* share substantial similarity in this region and in the putative zinc-binding domain of these proteins, the cysteine and histidine residues being conserved. Interestingly, both predicted protein sequences carry a very well conserved D34E motif common among all MULE elements as well as various transposases and integrases (Doak *et al.*, 1994; Lisch, 2002). This motif, which coordinates a metal ion, is known to be specifically required for the nicking and transesterification steps of the integration pathway (Haren *et al.*, 1999). The next closest homolog of *FAR1* and *FHY3* in the *Arabidopsis* genome is also shown for comparison.

The FAR1 protein is capable of activating the transcription of a reporter gene in transgenic Arabidopsis

Because FAR1 is a nuclear-localized putative signal transduction component, we suggested earlier that it might be a transcriptional regulator (Hudson *et al.*, 1999). As FAR1 is a homolog of transposases of the MULE family, it would therefore be a member of a new class of transcriptional regulators with transposase homology. The *MuDR* transposase MURA is known to bind DNA and to regulate both its own transcription and, occasionally, that of neighboring genes (Barkan and Martienssen, 1991; Benito and Walbot, 1997; Raizada *et al.*, 2001a,b). For this reason, we set out to investigate whether FAR1 could regulate the transcription of reporter genes. First, we studied transcriptional regulation by FAR1 in the yeast two-hybrid system. We fused FAR1 to the Gal4 DNA-binding domain and transformed a yeast strain carrying a Gal4 regulatory sequence upstream of a *Lac*Z reporter gene. FAR1 activated *Lac*Z transcription

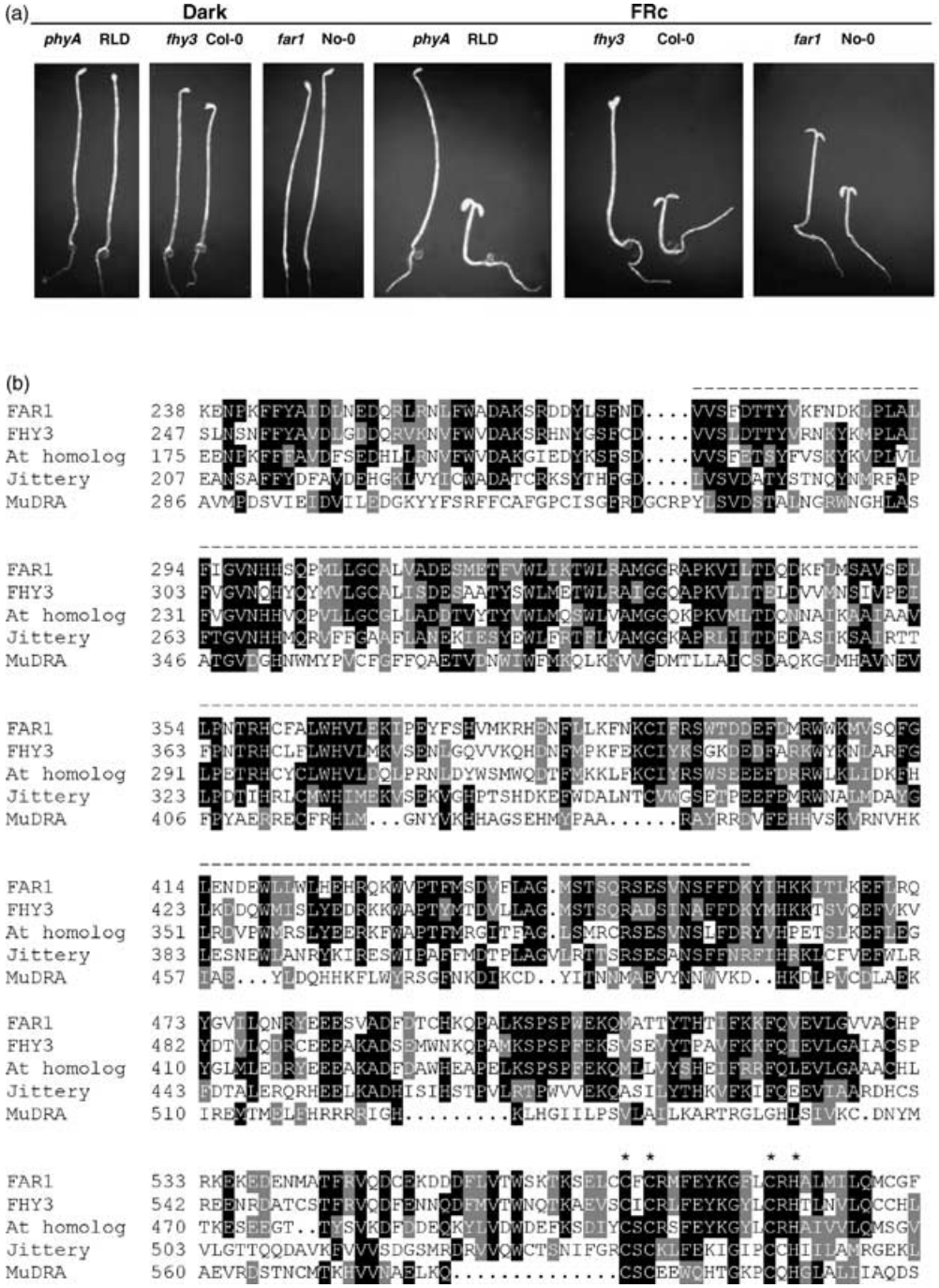

Figure 1. Phenotypes of the *far1* and *fhy3* mutants and sequences of the loci.
(a) Photographs of the plants used or mentioned in this study: *phyA* mutant and cognate RLD wild type, *fhy3* mutant and cognate Col-0 mutant, and *far1* mutant and cognate No-0 wild type. The plants were grown for 4 days in darkness (Dark) or for 24 h in darkness followed by 3 days in continuous far-red light (FRc).
(b) A partial PILEUP alignment of FAR1 with FHY3, a homologous protein (the closest homolog of FAR1 and FHY3 in the genome Acc. # AAF16668), the Jittery transposase (Acc. # AAF66982), and Mutator transposase A (MuDRA; Acc. # S59141). These proteins share substantial similarity throughout most of their length. This region includes the core Mutator transposase domain (indicated by dashes, conserved domain from pfam00872 (http://pfam.wustl.edu/cgi-bin/getdesc?acc=PF00872). The asterisks indicate the conserved cysteine and histidine residues that represent a potential zinc-binding motif.

by at least 15-fold, when expressed alone or together with a control vector. Enhanced activity was also observed when FAR1 fused with the GAL4 activation domain was co-expressed with the FAR1::binding-domain fusion protein, indicating a probable ability of FAR1 to homodimerize (M. Hudson and P.H. Quail, data not shown). Homodimerization is a common feature of transposases (Essers *et al.*, 2000). These results confirm those of Wang and Deng

(2002) who also demonstrated transcriptional activation and dimerization in these proteins using similar techniques.

We then examined the transcriptional activity of FAR1 in intact *Arabidopsis* seedlings under different light conditions. We developed a system of stably transformed *Arabidopsis* plants based on plants and constructs kindly supplied by Dr Ian Moore (Department of Plant Sciences, Oxford University, UK; Moore *et al.*, 1998; Figure 2a). We transformed a line carrying a *lac* operator sequence upstream of a GUS reporter with a second construct carrying FAR1 fused to the lacI DNA-binding domain described by Moore *et al.* (1998) as shown in Figure 2(a). We also made positive control plants carrying the Gal4 activation domain fused to the lacI DNA-binding domain, and negative controls carrying lacI alone. Ten lines of the positive control, and at least 20 of the negative control and the FAR1 fusion construct, were generated and screened for GUS activity in the T_2 generation by histochemical staining. Those seedlings that showed visible staining (seven positive controls, eight negative controls, and 16 FAR1::lac fusions, all in the pOP GUS reporter background) were selfed and made homozygous at both loci. We then assayed GUS activity in the seedlings of these lines after 4 days of growth in darkness, or 1 day in darkness followed by 3 days in far-red, red, or white light.

It can be seen from Figure 2(b) that, although the range of GUS expression levels in the lhFAR1 and lh lines overlap, on average, the levels in the lines carrying the FAR1::lac construct (lhFAR1) are consistently higher than the levels in the control lh lines, and the most active lhFAR1 lines have levels of GUS an order of magnitude higher than those in any of the negative controls. The positive control, lhG4, is considerably more active than lhFAR1. The difference between the lhFAR1 and lh sets of data is significant by inexact *t*-test ($P = 0.01$). When the most highly expressing

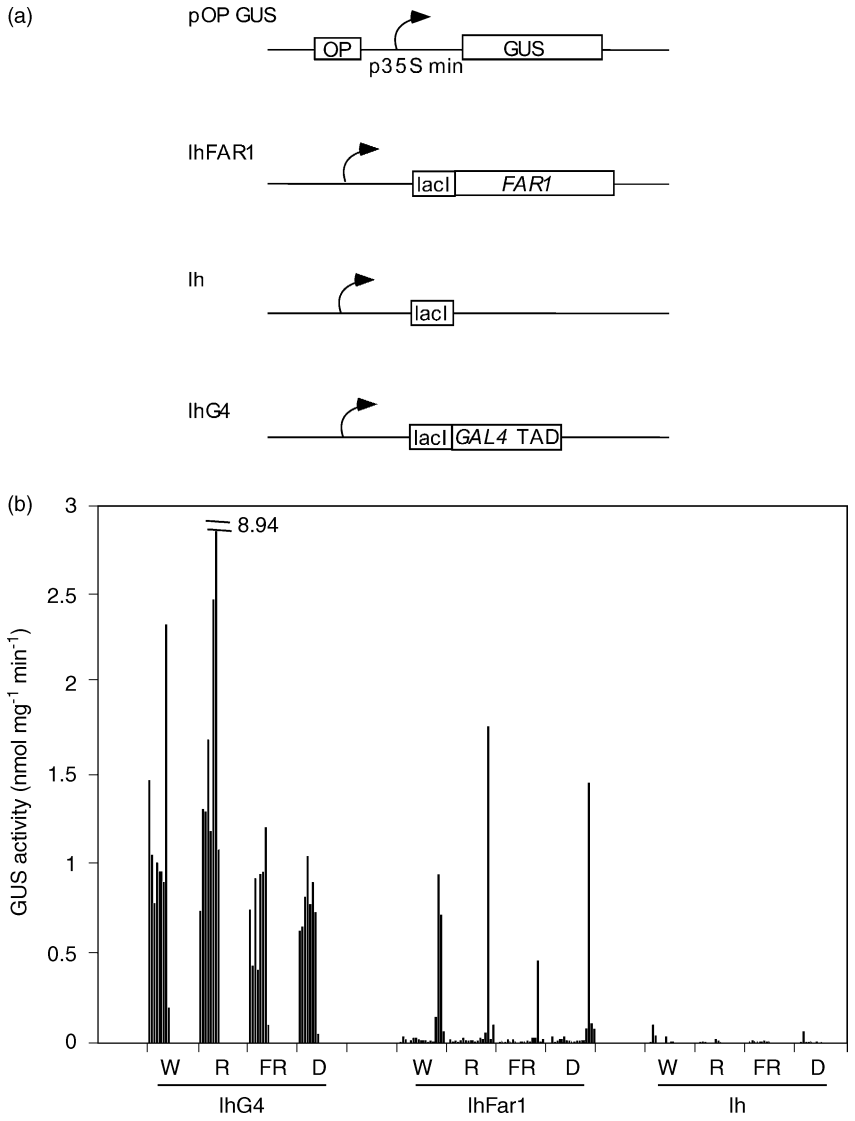

Figure 2. Transcriptional activation by FAR1.
(a) Diagrams to show constructs in transgenic plants in Part B. OP, *lac* operator sequence (lacI binding site); p35S min, minimal CaMV 35S promoter; lacI, DNA-binding domain of *Escherichia coli lac* repressor; *GAL4* TAD, transcriptional activation domain of GAL4 protein of yeast.
(b) GUS activities in transgenic plants carrying the pOP GUS reporter and one other construct described in Part A. Each symbol type for each construct represents the result of the fluorimetric GUS assay for a different transgenic line, grown in white light (W), continuous red light (R), continuous far red light (FR), or darkness (D). The difference between the lhFAR1 and lh sets of data is significant by inexact *t*-test ($P = 0.01$) even when the high-expressing outlier is excluded ($P = 0.04$).

FAR1 line visible in the figure is excluded from the analysis, the result is still significant ($P = 0.04$). As the pOP GUS reporter construct background is the same for all lines, and some lines of the lhFAR1 construct show strong activation of transcription, transcriptional activation is attributable to expression of the lacI::FAR1 fusion protein. However, we cannot identify any reproducible effect of light conditions on the transcriptional activity of FAR1.

A subset of genes, induced by continuous far-red light, is specifically affected in the far1 *and* fhy3 *mutants*

A number of transcripts that are strongly induced or repressed by phyA signaling have been defined previously (Tepperman *et al.*, 2001). In order to define the role of FAR1 and FHY3 in transcriptional regulation of genes controlled by the phyA pathway, we investigated the effect of the *far1* and *fhy3* mutant backgrounds on the far-red response of transcript levels. Using oligonucleotide microarrays, we quantified the levels of over 7000 transcripts after 1, 3, and 12 h of far-red light at 2.2 μmol m^{-2} sec^{-1}. Two dark controls were performed, at zero and 12 h into the experiment. Threefold replicate microarrays of the zero point and 1 h far-red point were performed.

We defined a set of light-induced genes in the following way. Genes with 'detectable' transcripts (defined by us as having at least one signal value above 25) were considered light induced if they showed a twofold increase in transcript level with respect to the mean of the three zero-hour dark control replicates and a 1.5-fold increase over the 12 h dark control after 1, 3, or 12 h of FRc. In the case of the 1 h time point, a *t*-test was performed to compare the dark, 1 h replicates of transcripts induced two- or more fold, and the *P*-value was required to be less than 0.05. In the case of the 3 and 12 h points, the point was required to be twofold above the upper 95% confidence limit of the dark control replicates in order to be considered reliable.

Using these criteria, we found 293 genes whose transcription was induced twofold in the Columbia ecotype, and 292 induced in the Nossen ecotype. Intriguingly, only 164 of these genes were common to the two lists (although many genes marginally failed to meet the twofold criteria in one ecotype). This was not completely unexpected because it is well established that there are strong, genetically determined differences in the responses of different *Arabidopsis* accessions to far-red light (Yanovsky *et al.*, 1997). However, there is a strong possibility that at least some of these genes are different as a result of experimental variation, particularly those with lower expression levels or those that may be affected by other conditions. It is not possible to distinguish between these possibilities using the data shown here, as our experiment was not designed to detect genetically determined differences. We therefore considered the behavior of each mutant–wild-type pair separately, using only the twofold induced genes that met our statistical criteria for robustness (above). Including genes that are more variable would compromise the effectiveness of the comparison between the mutant and the wild type. In accordance with the MIAME guidelines for microarray data disclosure, complete protocols used in the microarray experiments, lists of the light-induced genes in each ecotype, and the full, raw data for all the microarrays have been made available in the Supplementary Material.

We then compared the response to FRc in each of the light-induced genes for each ecotype between the wild type and the respective mutant. The comparison was achieved

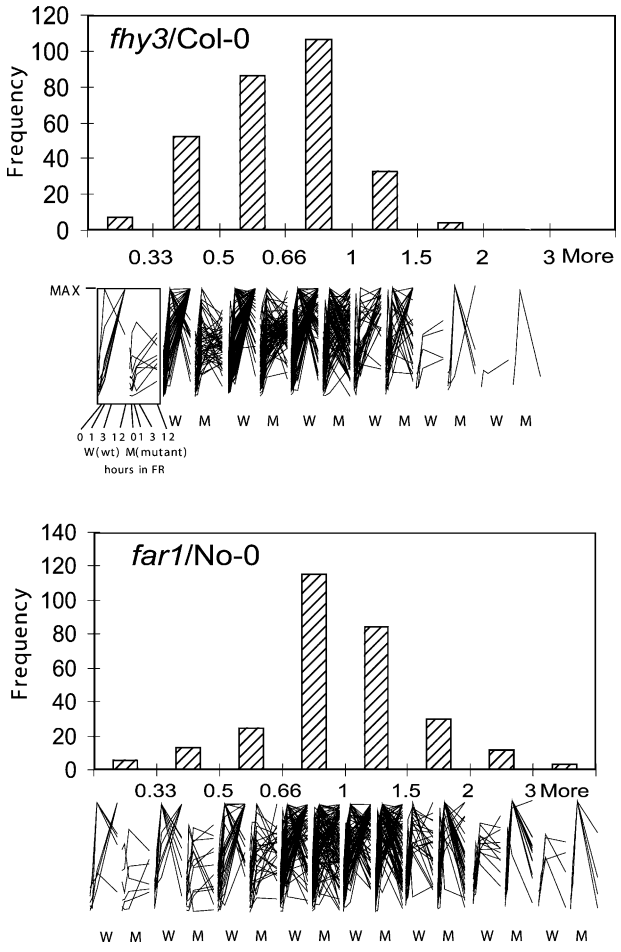

Figure 3. Light-induced gene expression in *fhy3* and *far1* mutants.
The histograms show the distribution of the ratio between the maximal light response in the mutant and that in the wild type, in the genes induced twofold or more in the wild type in response to far-red light. The ratio was obtained by dividing the maximum time point in the time course curve by the dark control point for the mutant and for the wild type, and by calculating the ratio of the two. The upper histogram shows the *fhy3*/Col-0 ratio and the lower the *far1*/No-0 ratio. A ratio of one indicates an identical response, 0.5 indicates a twofold greater response in the wild type than in the mutant, and two indicates a twofold greater response in the mutant than in the wild type. Below the histogram, the 12 h far-red light response curves of all the genes in each category are shown, normalized to fit the same axes. Details of individual genes are in the Supplementary Material.

Table 1 Genes under-induced in *fhy3*

fhy3 Fold ratio	*far1* Fold ratio	Affymetrix ID	TAIR ID	Annotation
0.12	0.36	16047	At2g25510	Unknown protein
0.12	0.22	17517	At5g36910	Thionin
0.24	0.51	18533	At5g65730	Xyloglucan endo-transglycosylase-like protein
0.24	1.11	12609	At3g44990	Xyloglucan endo-transglycosylase
0.25	1.37	19411	At2g37970	Unknown protein
0.26	2.40	11994	At2g39470	Unknown protein
0.31	0.38	17196	At4g28780	Proline-rich APG-like protein
0.32	1.11	19994	At4g18810	Putative protein
0.33	1.07	20050	At2g37660	Unknown protein
0.34	1.00	13808	At2g32640	Hypothetical protein
0.34	0.95	20648	At2g34460	Unknown protein
0.34	0.64	14393	At4g04840	Putative protein
0.35	0.46	16620	At5g57560	TCH4 protein
0.35	3.21	18255	At2g38860	Unknown protein
0.35	0.81	19017	At4g37800	Endo-xyloglucan transferase-like protein
0.36	0.71	14942	At4g17300	Asparagine – tRNA ligase
0.38	1.78	15490	At4g29590	Putative protein
0.39	0.62	14491	At2g43530	Putative trypsin inhibitor
0.40	1.54	15769	At4g31850	Putative protein (like crp1 protein, *Zea mays*)
0.40	0.84	18058	At1g24735	*S*-adenosyl-L-methionine:*trans*-caffeoyl-Coenzyme A 3-*O*-methyltransferase
0.41	1.00	18669	At4g17090	Putative beta-amylase
0.42	2.82	13954	At2g33250	Unknown protein
0.42	0.98	18052	At3g50820	Putative protein 1 photosystem II oxygen-evolving complex
0.42	1.17	16135	At3g02730	Thioredoxin f1
0.42	0.53	17024	At1g64900	Cytochrome P450, putative
0.43	2.59	16084	At1g05200	Putative ligand-gated ion channel protein
0.44	2.03	19127	At2g03750	Putative steroid sulfotransferase
0.44	0.82	14523	At2g43560	Putative FKBP type peptidyl-prolyl *cis–trans* isomerase
0.44	3.50	20017	At2g44290	Unknown protein
0.44	1.09	15986	At4g21280	Photosystem II oxygen-evolving complex protein 3-like
0.45	1.27	15108	At2g05100	Putative chlorophyll *a*/*b* binding protein
0.45	2.12	19187	At3g12110	Actin 11 (ACT11)
0.45	1.19	15093	At1g55480	Unknown protein (similar to putative tyrosine phosphatase)
0.45	1.92	12745	At2g21330	Putative fructose bisphosphate aldolase
0.45	1.95	16682	At5g44785	Unknown protein
0.45	2.15	14432	At4g27030	Putative protein
0.46	1.04	16613	At2g40540	Putative potassium transporter
0.46	0.85	17601	At4g36870	BEL1-like homeobox 2 protein (BLH2)
0.46	1.26	13798	At4g25910	Nitrogen fixation protein (like nitrogen fixation protein nifU – *Anabaena* sp.)
0.46	1.90	16856	At2g32540	Putative cellulose synthase
0.46	0.82	19708	At2g23420	Unknown protein
0.47	1.04	17841	At5g44870	Disease resistance protein-like
0.47	1.19	16503	At5g35630	Glutamate-ammonia ligase (EC 6.3.1.2) precursor, chloroplast
0.47	1.53	13284	At3g12580	Heat shock protein 70
0.47	1.30	15960	At3g09440	Heat shock protein (At-hsc70-3)
0.47	1.37	15676	At3g02830	Zinc finger protein 1 (zfn1)
0.47	0.85	18272	At2g40080	Unknown protein
0.48	0.77	14543	At2g24280	Putative prolylcarboxypeptidase
0.48	0.78	17087	At5g64040	Photosystem I reaction centre subunit psaN precursor
0.48	1.27	16548	At5g02120	One helix protein (OHP)
0.48	1.31	12962	At2g39190	Putative ABC transporter
0.48	2.48	14664	At1g78580	Trehalose-6-phosphate synthase, putative
0.48	1.48	18683	At5g05580	Temperature-sensitive omega-3 fatty acid desaturase, chloroplast precursor
0.49	1.43	16639	At1g69700	AtHVA22c; homolog of barley HVA22 (ABA-inducible DP1 homolog)
0.49	2.28	13003	At1g09240	Putative nicotianamine synthase
0.49	1.43	15496	At2g36800	Putative glucosyl transferase

Table 1 continued

Genes under-induced in *far1*

far1 Fold ratio	*fhy3* Fold ratio	Affymetrix ID	TAIR ID	Annotation
0.19	0.98	19720	At1g22690	Similarity to gibberellin-regulated protein 2 precursor (GAST1)
0.22	0.12	17517	At5g36910	Thionin
0.23	2.63	20322	At5g14130	Peroxidase ATP20a
0.29	1.22	14439	At1g32560	Late-embryogenesis abundant protein
0.30	0.83	17648	At5g65890	Uridylyl transferases-like
0.30	1.27	20450	At5g24150	Squalene monooxygenase
0.34	1.51	19548	At2g15050	Putative lipid transfer protein
0.36	4.00	16966	At2g16850	Putative plasma membrane intrinsic protein (aquaporin)
0.38	0.31	17196	At4g28780	Proline-rich APG-like protein
0.41	0.84	13672	At5g11060	Homeobox protein; KNOTTED-1 LI4 (KNAT4)
0.44	0.56	14868	At2g31560	Unknown protein
0.46	0.35	16620	At5g57560	TCH4 protein (xyloglucan endotransglycosylase)
0.46	3.01	18755	At4g25780	Putative pathogenesis-related protein
0.48	2.14	15327	At4g38300	Putative tapetum-specific protein
0.48	0.81	16004	At3g54890	Chlorophyll *a*/*b*-binding protein
0.48	0.96	19944	At1g67360	Stress-related protein
0.48	0.68	19998	At2g30520	Unknown protein
0.48	2.53	20227	At1g52030	Myrosinase binding protein, putative

Transcripts that show a twofold or more reduced transcript-level induction by light in the *fhy3* or *far1* mutants. The 'fold ratio' value is the ratio between the maximum fold induction observed at any time point in the mutant divided by the maximum fold induction in the isogenic wild type, rounded to two decimal places. See Supplementary Material for the full data set and criteria.

by dividing the maximum fold response (at any time point) in the mutant with the maximum fold response of the wild type, giving a 'max fold ratio'. A histogram was plotted to demonstrate the differences in response between the wild type and the mutant in different genes (Figure 3). The data show that many genes are induced normally, or close to normally, by FRc in both mutants (i.e. the max fold ratio is between 0.66 and 1.5). The distribution of response in the *fhy3* mutant is, however, strongly skewed towards the left-hand side of the graph, showing that 85% of light-induced genes respond less strongly in *fhy3* than in the Col-0 wild type.

In the case of most genes, the effect of the *fhy3* mutation on light induction is subtle. Most of the light-regulated genes fall below a max fold ratio of 1 (indicating a reduced response), but only 63 fall below a max fold ratio of 0.5 (indicating that these genes respond at least twofold more strongly in the wild type than in the mutant). Consequently, the light response of the subset of genes showing a twofold reduced response is specifically, and strongly, dependent on *fhy3*. A complete list of these genes and their functional annotation is given in Table 1, and the full list of all light-induced genes and the effect of the mutation on their response is available in the Supplementary Material. Note that only one of the early light-induced transcription factors described by Tepperman *et al.* (2001), ZF1, shows a twofold reduced light response in *fhy3* (see Figure 5a). The most strongly affected genes are induced later and more strongly by FRc. A number of these genes appear to be possibly involved in the elongation and growth of cells, such as xyloglucan endotransglycosylases, and members of a family of proline-rich proteins showing homology to GDSL motif lipases and hydrolases. A small number of genes show an enhanced light response in the mutant, possibly because of a feedback mechanism. (Details of the genes showing all levels of response in the mutants are available in the Supplementary Material).

The *far1* mutant has a much weaker effect on light-induced gene expression. Nonetheless, the histogram of differential transcript-light responses between *far1* and the No-0 wild type is also somewhat skewed to the left. Some genes are strongly reduced in their response to far-red light in the *far1* mutant relative to the wild type. Their details are given in Table 1; the complete set of light-induced genes and their differential responsiveness are detailed in the Supplementary Material. Comparing the lists of genes affected in their light responsiveness between the *far1* and *fhy3* mutants, we observe that some genes, for example those encoding thionin and a proline-rich protein are affected in a similar way in both mutants. Other transcripts appear to be specifically affected by one of the mutant loci. We consider those genes affected in a similar way in both mutant backgrounds to be the most likely candidates for involvement in the creation of the similar mutant phenotypes (see later in this section).

The light-repressed genes were also investigated in a similar manner. A set of twofold light-repressed genes was defined for each ecotype. These genes were repressed twofold at 1, 3, or 12 h, relative to the mean of the three dark control replicates, and 1.5-fold relative to the 12 h dark

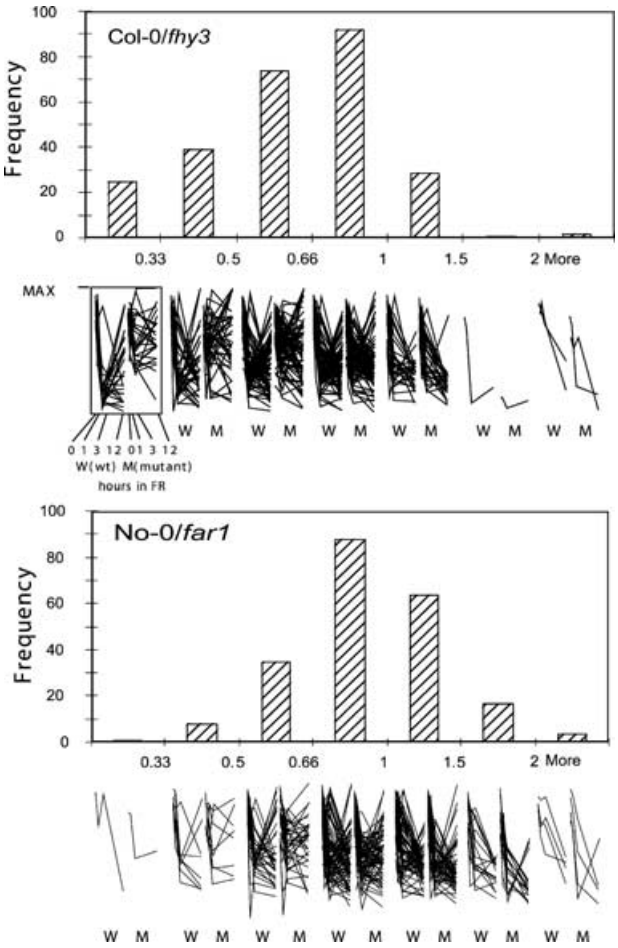

Figure 4. Light-repressed gene expression in *fhy3* and *far1* mutants.
The histograms show the distribution of the ratio between the maximal light response in the wild type and that in the mutant, in the genes repressed twofold or more in the wild type in response to far-red light. The ratio was obtained by dividing the minimum time point in the time course curve by the dark control point for the wild type and for the mutant, and by calculating the ratio of the two. The upper histogram shows the Col-0/*fhy3* ratio and the lower the No-0/*far1* ratio. A ratio of one indicates an identical response, 0.5 indicates a twofold greater response in the wild type than in the mutant, and two indicates a twofold greater response in the mutant than in the wild type. Below the histogram, the 12 h far-red light response curves of all the genes in each category are shown, normalized to fit the same axes. Details of individual genes are in the Supplementary Material.

control. To ensure that the dark control level was consistently measurable, the dark point replicates were required to have a lower 95% confidence interval of 25 or higher. The differences between ecotypes for the light-repressed genes were even more substantial than those for the light-induced gene sets. Of the 257 transcripts found to meet these criteria in Columbia, and 195 in Nossen, only 69 were common to both. It should be noted, however, that repression of transcript levels is subject to a great deal of experimental variation as a result of the many factors involved in determining mRNA stability, and that this could be the cause of some of the observed variation.

Again, the maximal response of the wild type and the mutant was determined and a response ratio calculated. The data in Figure 4 demonstrate that as with light-induced genes, the majority of light-repressed genes respond less strongly in the *fhy3* background than the wild type, and a large number of genes have a response reduced twofold or more. In the *far1* background, a significant, but smaller, loss of response is visible in some genes. A complete list of those genes showing a twofold or more reduced response in either mutant background is given in Table 2. Full details of the behavior of all light-repressed genes studied are given in the Supplementary Material.

The far1 *and/or* fhy3 *mutations specifically affect light induction of some transcription factors and potential target genes*

A previously published proposed pathway for the regulation of photomorphogenesis by light signals perceived by phytochromes is that photoperception is followed by the initiation of a transcriptional cascade. This cascade is initiated by transcription factors whose expression levels respond rapidly to light signals (Tepperman *et al.*, 2001). We therefore examined the possibility that the postulated signal transduction mutations investigated here may affect the light induction of the early light-induced transcription factors described by Tepperman *et al.* (2001). Note that the profiles of these key regulatory genes shown in Figure 5(a) are extremely consistent between the two ecotypes, despite the large variations seen in the response of many other transcripts.

We found that the majority of the far-red-light-induced transcription factors showed responses close to the wild type in both mutant backgrounds (Figure 5a). In six cases, the transcript induction profile is visibly (but in all but one case less than twofold) affected by the mutations. For example, the transcripts for the AP2 domain protein (AP2D), the zinc finger proteins 1 and 5 (ZF1 and ZF5), and the MYB-106 protein (MYB-106) are significantly reduced in the extent of their response by one or both mutations. We interpret genes such as these, which show a loss of response in both mutants, as the most likely to be involved in the processes determining the related photomorphogenic phenotypes of the mutants (especially those where the *fhy3* mutation has the stronger effect).

In the case of some transcription factor genes, induction is stronger in one or both mutants than in wild type. Examples of this are transcripts for LHY, constans (CO) and H-promoter binding factor 2a (DOF). It is also of interest that, in some cases, a somewhat altered level of transcript is observed at the dark control point. These observations may indicate a perturbation in general transcriptional regulation in both mutants (see later in Results section and Figure 6).

Table 2 Genes under-repressed in *fhy3*

fhy3 Fold ratio	*far1* Fold ratio	Affymetrix ID	TAIR ID	Annotation
0.09	0.76	20043	At4g31080	Putative protein
0.17	2.00	14572	At5g35100	Putative protein
0.18	0.81	12650	At2g29910	Hypothetical protein
0.19	6.54	20641	At1g52690	Similar to late embryogenesis-abundant protein (*Brassica napus*)
0.2	1.83	15487	At2g38000	Unknown protein
0.22	2.60	16279	At2g04570	Putative GDSL-motif lipase/hydrolase similar to APG proteins
0.23	1.54	14806	At2g25280	Hypothetical protein
0.23	1.43	15977	At2g45960	Aquaporin (plasma membrane intrinsic protein 1B)
0.23	0.67	16096	At5g15150	Homeobox-leucine zipper protein HAT7
0.23	0.99	19977	At3g48360	Putative protein
0.25	1.14	13781	At2g18010	Putative auxin-regulated protein
0.25	1.53	15519	At1g03090	Putative 3-methylcrotonyl-CoA carboxylase
0.26	1.47	13618	At2g05990	Enoyl-ACP reductase (enr-A)
0.27	0.47	19118	At4g12550	Putative cell wall-plasma membrane disconnecting CLCT protein
0.27	1.82	19945	At2g17880	Putative DnaJ protein
0.29	1.16	15488	At3g52220	Unknown protein
0.29	0.95	19374	At2g28670	Putative disease resistance response protein
0.3	1.61	13551	At2g42880	Putative MAP kinase
0.3	1.69	13918	At1g54710	Hypothetical protein
0.31	1.65	18625	At1g03290	Unknown protein
0.32	1.44	12227	At1g13990	Unknown protein
0.32	3.04	16038	At5g66400	Dehydrin RAB18-like protein
0.32	0.80	18384	At2g45430	Putative AT-hook DNA-binding protein
0.33	1.60	11992	At1g24160	Unknown protein
0.33	1.25	12627	At2g31800	Putative protein kinase
0.33	0.92	19594	At3g21770	Putative peroxidase
0.33	0.55	19914	At2g28890	Unknown protein
0.34	1.51	12592	At4g35570	HMG delta protein
0.35	0.70	14028	At4g02290	Putative endo-1,4-beta glucanase
0.36	1.19	14924	At2g28400	Hypothetical protein
0.37	1.95	14996	At4g38440	Putative protein
0.37	1.52	17722	At2g38820	Hypothetical protein
0.37	1.48	18983	At4g12510	pEARLI 1-like protein
0.38	1.31	12660	At4g25710	Putative protein
0.38	0.89	16144	At2g18170	MAP (mitogen activated protein) kinase-like
0.39	2.00	14539	At2g42490	Putative copper amine oxidase
0.39	1.42	17961	At1g01120	Fatty acid elongase 3-ketoacyl-CoA synthase 1
0.39	0.54	19626	At2g35780	Putative serine carboxypeptidase II
0.39	1.25	20100	At2g32000	Putative DNA topoisomerase III beta
0.41	2.33	12426	At2g21880	Putative RAS superfamily GTP-binding protein
0.41	0.98	19154	At2g23980	Cyclic nucleotide and calmodulin-regulated ion channel
0.41	1.59	19691	At1g31070	UDP-*N*-acetylglucosamine pyrophosphorylase-like protein
0.42	0.63	20494	At2g42570	Unknown protein
0.43	1.09	17555	At2g33860	Auxin response transcription factor 3 (ETTIN/ARF3)
0.43	2.26	19877	At3g48390	Putative apoptosis gene MA3
0.43	0.93	20053	At1g60610	Unknown protein
0.44	2.00	15145	At1g02200	Similar to receptor-like protein glossy1 (gl1)
0.44	0.92	19135	At2g38750	Putative annexin
0.45	1.94	15520	At4g27760	Forever young gene (FEY)
0.45	0.86	16494	At2g47940	DegP2 protease
0.46	1.24	12026	At4g26550	Probable membrane protein
0.46	0.97	14550	At4g23690	Putative disease resistance response protein
0.46	1.38	17715	At2g46560	Hypothetical protein
0.46	0.80	19464	At2g01450	Putative MAP kinase
0.46	1.46	19688	At4g25580	Similarity to low-temperature-induced protein 65
0.47	0.87	18967	At3g51920	Putative calmodulin
0.47	3.82	19152	At5g06760	Late embryogenesis abundant protein LEA like
0.48	1.40	13549	At1g23800	Putative aldehyde dehydrogenase
0.48	2.06	16528	At1g27130	Glutathione transferase, putative
0.48	1.12	19841	At1g11670	Unknown protein
0.48	1.08	20152	At4g19860	Putative protein

Table 2 continued

Genes under-repressed in *far1*				
far1 Fold ratio	*fhy3* Fold ratio	Affymetrix ID	TAIR ID	Annotation
0.31	0.97	14101	At4g33640	Putative protein
0.41	0.75	15933	At1g21830	Unknown protein
0.46	1.06	14062	At2g47780	Unknown protein
0.46	1.30	18224	At4g21830	Putative protein
0.47	0.85	13101	At4g28470	Putative protein
0.47	0.67	19118	At4g12550	Putative cell wall-plasma membrane disconnecting CLCT protein
0.49	1.14	12538	At1g11580	Putative pectin methylesterase
0.49	1.24	20336	At2g29730	Putative flavonol 3-*O*-glucosyltransferase
0.5	1.08	13387	At5g27960	Putative MADS box protein AGL29

Transcripts that show a twofold or more reduced transcript level repression by light in the *fhy3* or *far1* mutants. The 'fold ratio' value is the ratio between the maximum fold repression observed at any time point in the isogenic wild type divided by the maximum fold repression in the mutant, rounded to two decimal places. See Supplementary Material for the full data set and criteria.

Some examples of other genes affected in their light induction are shown in Figure 5(b). Note that these represent transcripts that are affected unusually strongly in the mutants, and hence are not representative; an impression of the effect on the majority of light-regulated genes can be better gained from Figures 3 and 4, and the Supplementary Material. The induction of the thionin gene shown is very strongly affected by both mutations, while the thioredoxin F1 gene (TF1) also shows reduced induction in both. This may indicate that *far1* and *fhy3* are especially important for the light regulation of transcripts for redox-related proteins. The proline-rich APG-like protein (P-rich) and the xyloglucan endotransglycosylase (XEG) are examples of the putative cell-wall-related proteins whose transcripts also seem to be strongly affected by these mutations. Both of these genes are members of gene families, of which others are affected in a similar way. Note that the light induction responses of xyloglucan endotransglycosylases show considerable variation between ecotypes and experiments; this is to be expected of genes that are regulated by many different environmental stimuli (Xu *et al*., 1995). As a group, however, they seem to respond strongly to light, and to display a reduction of their light induction in the mutants. The ACC oxidase shown (ACC-O) is affected by both mutations, but the differential response falls just short of twofold. These data are therefore omitted from Table 1, but all such profiles are available from the Supplementary Material.

The far1 *and* fhy3 *mutants display a light-independent transcriptional phenotype*

One aspect of the molecular phenotype of *far1* and *fhy3* is the reduction in light-induced transcriptional responses described above. Another consequence of these mutations is a light-independent alteration in levels of several transcripts. These transcript level anomalies were detected by comparing replicates of dark and 1 h far-red treatments in the mutants and wild type, using the *t*-test to discover statistically significant differences. Our criteria require that the mean transcript level in the dark-grown seedlings be altered by at least twofold in either mutant, that the dark replicates of mutant and wild type are significantly different ($P < 0.05$), and that the mean expression level is clearly detectable in either the mutant or the wild type (details in the Supplementary Material). We found that several genes were reduced in their transcript levels in the *fhy3* mutant irrespective of FRc treatment, a phenomenon noted by Desnos *et al*. (2001) with respect to the *FHY1* gene in the *fhy3* mutant background. The profiles of these genes are shown in Figure 6(a); most are not strongly regulated by light and are absent from the set of light-regulated genes defined by Tepperman *et al.* (2001), although many do show slight light induction or repression. Details of these genes are available in Table 3. We re-examined the *phyA* mutant data of Tepperman *et al*. (2001) to investigate the effect of the *phyA* mutation on the level of the transcripts, which are identified here as affected by the *fhy3* or *far1* mutations in the dark. None of these genes, which showed significantly lower expression in *far1* or *fhy3*, did so in the *phyA* mutant (data not shown). We did identify that one of the genes identified by Tepperman *et al*. (2001) as phyA responsive was also affected by the *phyA* mutation in darkness (At1g05260, a putative peroxidase, shows 2.1-fold increased levels in the dark-grown *phyA* mutant). However, this gene did not show significant dark effects in *far1* or *fhy3*. Raw (i.e. not normalized) data for our replicate points are shown in Figure 6(b) for some representative probe sets from this class. The full set of genes shown in Table 3 indicates that most of the transcripts showing reduced levels in one mutant also show reduced levels in the other, although often the difference in one mutant did not reach the arbitrary twofold cut-off value. Notable exceptions to this are the genes where the values for one mutant are increased, whereas the values for the other are reduced; many of the most strongly affected transcripts fall into this

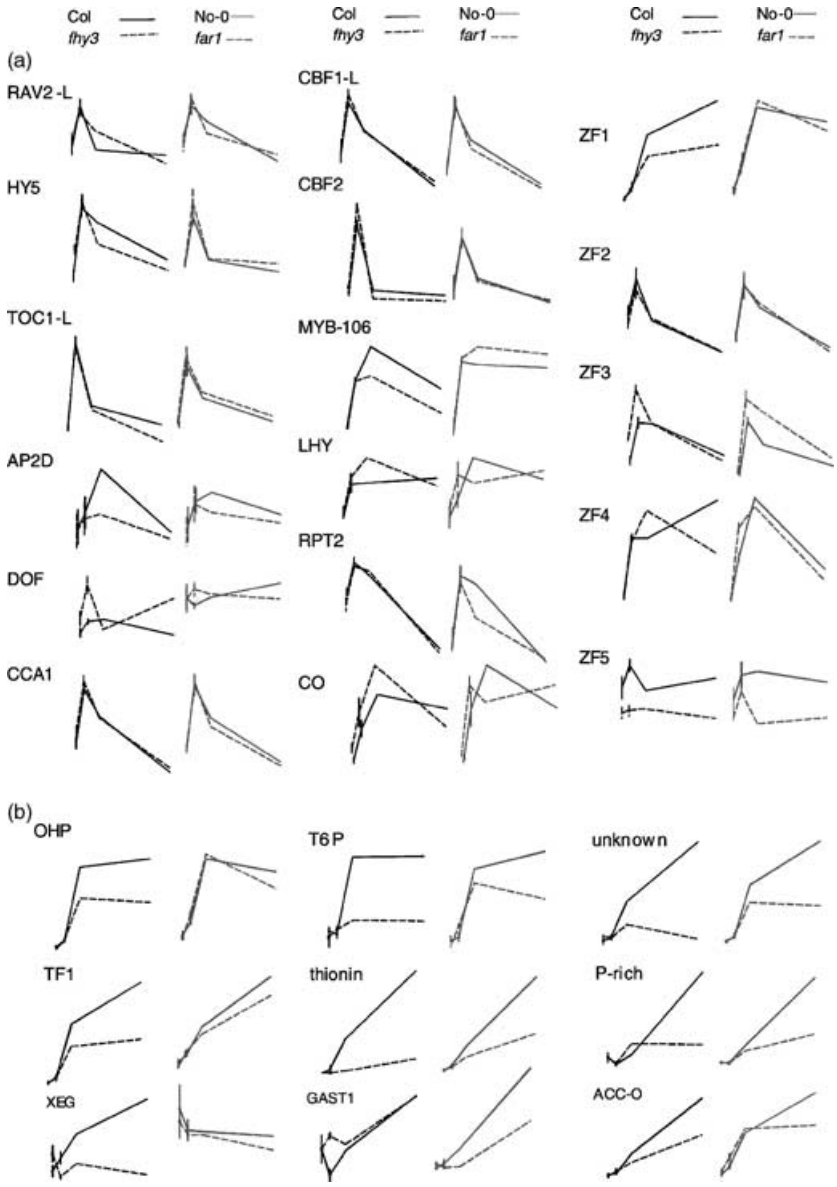

Figure 5. Comparison of light-induced gene behavior in the *far1* or *fhy3* mutant relative to the wild type.
(a) Putative transcription factor genes, which respond rapidly to light, compared between the *far1* and *fhy3* mutants and their relative wild types. The left-hand graph for each transcript shows wild-type Columbia and *fhy3* mutant; the right-hand graph shows wild-type Nossen and *far1* mutant. Each graph shows transcript signal values at zero, 1, 3, and 12 h after transfer to continuous far-red irradiation. Error bars on the replicated zero- and one-hour points represent standard errors of the mean. Mutants are shown in dashed lines and wild types in solid. ZF5, zinc finger protein 5 (At5g59820); RAV2-L, related to ABI3/VP1-2-like (At2g36080); HY5, HY5 (At5g11260); TOC1-L, timing of CAB1-like (At2g46790); AP2D, putative AP2-domain transcription factor (At2g28550); ZF1, zinc finger protein 1 (At3g02830); DOF, H-protein promoter binding factor-2a (At3g47500); CCA1, circadian clock associated protein 1 (At2g46830); CBF1-L, CRT/DRE-binding factor 1-like (At4g25480); CBF2, CRT/DRE binding factor 2 (At4g25470); MYB-106, putative MYB-related transcription factor (At5g15310); LHY, late elongated hypocotyl (At1g01060); RPT2, root phototropism 2 (At2g30520); CO, constans (At5g15850); ZF3, putative constans-like b-box zinc finger protein (At2g31380); ZF4, zinc finger protein 4 (At4g38960); ZF2, constans-like zinc finger protein (At1g06040). The WRKY6-like transcription factor was not found to be light responsive in these experiments (data not shown).
(b) Examples of light-induced genes strongly affected by the *far1* or *fhy3* mutations. The graphs are arranged as in (a). OHP, one helix protein (At5g02120); T6P, trehalose 6-phosphate synthase (At1g78580); unknown, putative protein (At4g04840); TF1, thioredoxin F1 (At3g02730); thionin, thionin (At5g36910); P-rich, proline-rich APG-like protein (At4g28780); XEG, xyloglucan endo-transglycosylase (At3g44990); GAST1, similarity to gibberellin-regulated protein 2 precursor (GAST1; At1g22690); ACC-O, putative 1-aminocyclopropane-1-carboxylate oxidase (At1g04350).

category including the transcript for the FLF MADS box protein. Note that transcription factors (genes for CAAT/EBP and two genes for MADS box proteins) are among the genes showing reduced expression in *fhy3* and/or *far1*. As the *FHY1* transcript was not represented by a probe on our microarray, we performed replicate RNA gel blots to determine the relative levels of this transcript. These data are indicated with an asterisk in Figure 6(b).

Discussion

Transposase homology

The *Arabidopsis* genome contains a large number of Mutator-like elements (MULEs) (Feschotte *et al.*, 2002; Lisch *et al.*, 2001). The *ddm1* mutant, which shows reduced levels of DNA methylation, has increased levels of transposition. Some of the MULEs in *Arabidopsis* are active in this mutant, showing that they are functional transposons (Singer *et al.*, 2001). The sequence homology evidence shown here gives strong support to the proposition that the common ancestor of *FAR1* and *FHY3* was a mobile element of the MULE type. The *FAR1* and *FHY3* genes are stably integrated into the genome, however, and show no detectable sign of terminal inverted repeats or other transposon-like structures. They are present in regions of single-copy, expressed genes and are not surrounded by other transposon homologs. In contrast, many of the MULEs in *Arabidopsis* are present in regions of heterochromatin such as that described by the CSHL/WUGSC/PEB, Arabidopsis Sequencing Consortium (2000). Neither *FAR1* nor *FHY3* appear to be in such a region. For these reasons, we believe that *FAR1* and *FHY3* have not been capable of acting as transposons for a substantial period of evolutionary time. We suggest that *FAR1* and *FHY3* are stable, expressed, genomic homologs of the *MuDRA*-component of MULEs. The human genome also contains what appear to be stably integrated and tissue-specifically expressed transposase-like genes, but no function has yet been ascribed to them (Lander *et al.*, 2001).

Transcriptional regulation

It is well established that transposase expression can regulate the expression of genes close to a related transposon, or a TIR fragment (Barkan and Martienssen, 1991; Martienssen *et al.*, 1989). Transposases of the *Mutator* family are known to directly bind DNA and regulate the expression of their own genes and genes into which a MULE element has been inserted (Barkan and Martienssen, 1991; Benito and Walbot, 1997; Raizada *et al.*, 2001b). This regulation is associated with changes in methylation status of *Mu* TIRs that occur when active (i.e. transposase-expressing) *Mu* elements are present (Walbot *et al.*, 1988). Thus, *Mutator* elements are capable of controlling gene expression, almost certainly via the expression of the MuDRA transposase. Transposase mediation of changes in gene expression can involve a variety of mechanisms, but, in each case, the expression of a gene can become dependent on the presence or absence of the transposase because of the presence of a portion of the transposon. It has been hypothesized that this form of gene re-programming could represent an important source of evolutionary change (Kidwell and Lisch, 1997).

The transcriptional and/or DNA-methylation control functions of a transposase like *Mutator* may have been retained and modified by evolutionary sequence changes that otherwise resulted in the loss of DNA excision and integration activity. Such an immobilized transposase-derived transcription factor could then become of selective advantage to the organism by means of a capacity to regulate genes close to any TIR sequences (acting as *cis*-regulatory elements) to which it was still able to bind. Co-evolution of the MULE-like transcription factor gene and the TIR-like recognition site of its product could eventually produce a system of *cis* elements with only distant similarity to TIRs (which are, in any case, highly variable) and transposases. This proposed sequence of events bears some similarities to that involved in the evolution of the human immune system. In that case, the *RAG1* and *RAG2* genes are hypothesized to have been a part of an ancestral transposon that became 'domesticated'(Agrawal *et al.*, 1998). Their recombinational function was retained, but the original TIRs surrounding the genes were lost over time. The targets of the proteins encoded by *RAG1* and *RAG2* were short sequences that now flank portions of the immunoglobin genes and serve as recombination signal sequences.

The transcriptional activation activity displayed by the FAR1 protein in both yeast and *Arabidopsis* provides evidence for potential involvement in transcriptional regulation. We have identified a number of potential target genes for FAR1 and FHY3. We suggest that FAR1 and FHY3 may directly control the expression of some or all of these genes by means of relic TIR sequences acting as *cis*-regulatory elements in the DNA sequence of the target genes. Unfortunately, that portion of the ancestral TIR necessary for binding the transposase may have been only a portion of the TIR, and its identity may now be obscured as a result of mutational changes in the rest of the TIR. However, the promoter of the FLF gene (the most strongly affected transcript in the FHY3 mutant) contains the sequence:

Figure 6. Genes with overall reduced expression in the *far1* or *fhy3* mutant relative to the wild type.
(a) Normalized time course (12 h) profiles of the genes found to have twofold or more reduced transcript levels in dark-grown *fhy3* or *far1* mutants. The normalized average profile is given by the bold dashed line. Note that most of these genes are not light regulated under these conditions. Details of individual genes are in the Supplementary Material.
(b) Examples of representative genes in (a) are shown without normalization using the mean of the expression values in dark-grown seedlings (black bars) and the mean of the expression values of seedlings treated with far-red light for 1 h (striped bars). Values are the mean of three replicates, and error bars are standard errors of the mean. *Profile of the expression of the *FHY1* gene is expressed as above except that the values are derived from Northern blotting as a proportion of the signal from the 18S ribosomal transcripts.

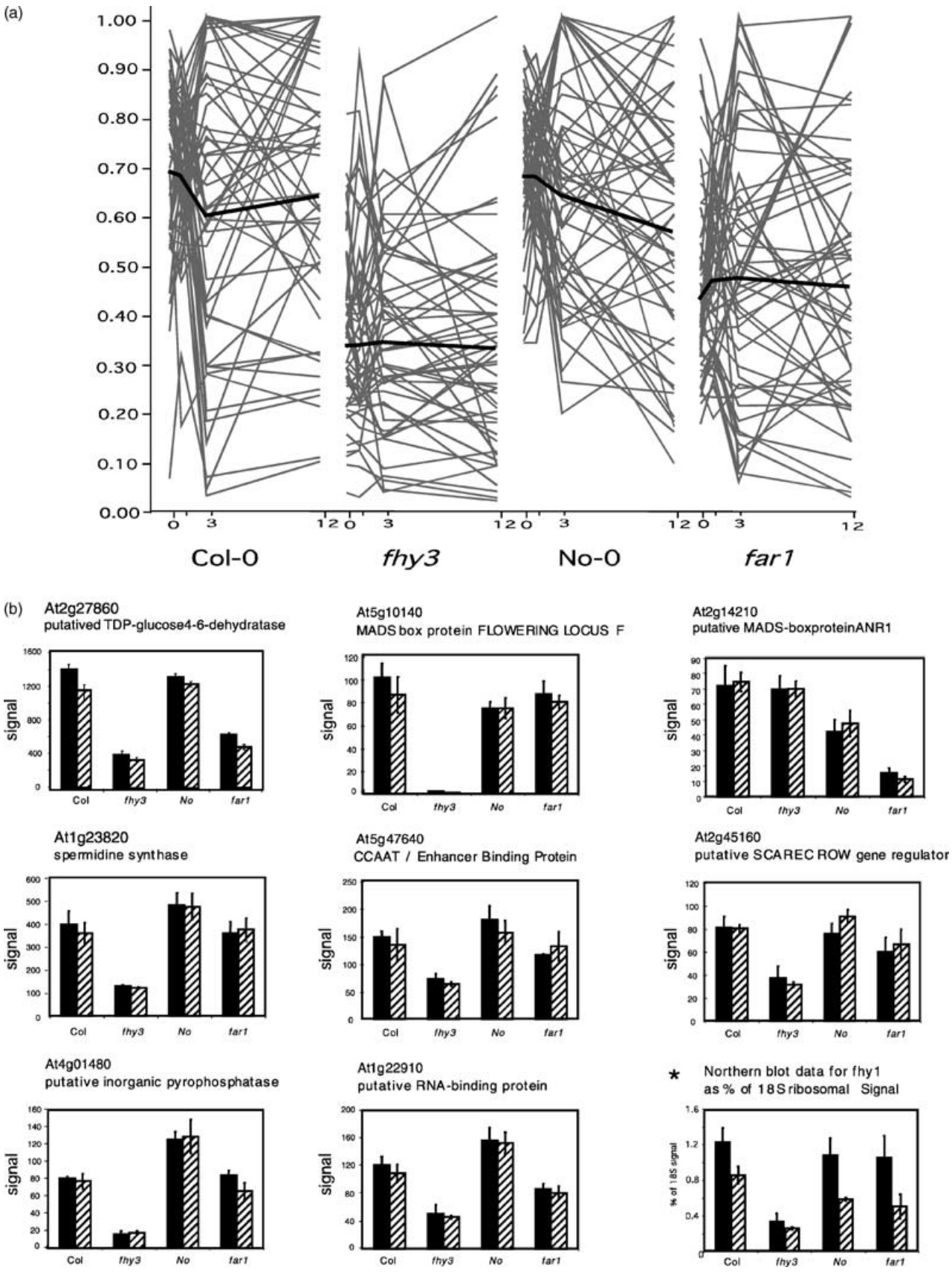
(a)
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0 3 12
0 3 12
0 3 12
0 3 12
Col-0
fhy3
No-0
far1
(b)
At2g27860
putatived TDP-glucose4-6-dehydratase
At5g10140
MADS box protein FLOWERING LOCUS F
At2g14210
putative MADS-boxproteinANR1
At1g23820
spermidine synthase
At5g47640
CCAAT / Enhancer Binding Protein
At2g45160
putative SCAREC ROW gene regulator
At4g01480
putative inorganic pyrophosphatase
At1g22910
putative RNA-binding protein
* Northern blot data for fhy1
as % of 18S ribosomal Signal
signal
Col
fhy3
No
far1

Table 3 Transcripts under-expressed in darkness in *fhy3* relative to wild type

fhy3 Fold reduction	*far1* Fold reduction	Affymetrix ID	TAIR ID	Annotation
41.55	0.86	17501	At5g10140	MADS box protein FLOWERING LOCUS F (FLF)
9.28	0.90	15188	At2g41740	Putative villin 2
5.47	0.98	18927	At1g52100	Jasmonate inducible protein, putative
4.93	1.48	17953	At4g01480	Putative inorganic phosphatase
4.63	2.26	20719	At4g11190	Putative disease resistance response protein
4.54	2.18	12748	At4g11320	Drought-inducible cysteine proteinase RD21A precursor -like protein
3.57	2.08	13089	At2g27860	Putative dTDP-glucose 4-6-dehydratase
3.54	1.31	17832	At2g16060	Class 1 non-symbiotic hemoglobin (AHB1)
3.22	1.27	20245	At2g47800	Glutathione-conjugate transporter AtMRP4
3.16	1.08	14556	At4g35920	Putative protein
3.08	1.88	13509	At4g04210	Putative membrane trafficking factor
3.05	1.34	12811	At1g23820	Putative spermidine synthase
2.91	1.20	19184	At1g27030	Unknown protein
2.76	2.77	18351	At4g23060	Putative protein SF16 protein
2.64	1.19	14779	At2g30010	Hypothetical protein
2.38	1.81	19012	At1g22910	Putative RNA-binding protein
2.35	1.38	17310	At3g51810	Embryonic abundant protein AtEm1
2.30	1.25	20636	At4g17870	Putative protein
2.28	1.37	17560	At2g28950	Expansin AtEx6
2.19	2.19	12162	At2g42900	Unknown protein
2.18	1.26	20699	At2g45160	Putative SCARECROW gene regulator
2.17	1.07	12460	At2g15400	DNA-directed RNA polymerase II, third largest subunit
2.17	1.33	20051	At1g08940	Unknown protein
2.16	1.61	12330	At2g34080	Cysteine proteinase
2.13	1.41	16362	At2g33050	Putative leucine-rich repeat disease resistance protein
2.11	1.22	12587	At2g22770	Putative bHLH transcription factor
2.11	0.97	14956	At2g18440	Unknown protein
2.09	1.01	13589	At1g09740	Putative ER6 protein
2.07	0.83	15374	At2g03460	Unknown protein
2.07	1.30	18421	At2g35630	Similar to ch-TOG protein from *Homo sapiens*
2.03	1.55	20437	At5g47640	Strong similarity to CCAAT-box-binding trancription factor
2.00	1.62	13144	At2g35860	Unknown protein

Transcripts under-expressed in darkness in *far1* relative to wild type

far1 Fold reduction	*fhy3* Fold reduction	Affymetrix ID	TAIR ID	Annotation
4.39	0.17	13198	At4g28520	12S Cruciferin seed storage protein
2.85	4.83	18852	At2g25160	Putative cytochrome P450
2.77	2.76	18351	At4g23060	Putative protein
2.7	1.04	19609	At2g14210	Putative MADS-box protein ANR1
2.45	0.84	20345	At4g01700	Putative chitinase
2.43	1.08	15365	At2g41990	Unknown protein
2.33	1.18	20418	At1g11600	Putative cytochrome P450
2.28	0.83	16991	At4g25630	Fibrillarin 2 (AtFib2)
2.24	2.46	13048	At2g02850	Putative basic blue protein (plantacyanin)
2.24	1.55	19459	At4g36880	Cysteine proteinase
2.22	0.93	13539	At3g47380	Putative protein pectinesterase homolog
2.19	2.19	12162	At2g42900	Unknown protein
2.18	4.54	12748	At4g11320	Similarity to cysteine proteinase RD21A (thiol protease)
2.17	1.30	15348	At1g15470	Similar to serine-threonine kinase receptor-associated protein
2.16	1.48	19722	At5g25810	Transcription factor TINY
2.12	1.24	20154	At4g33120	Putative cyclopropane-fatty-acyl-phospholipid synthase
2.1	1.27	14722	At5g10450	14-3-3 Protein GF14lambda
2.09	0.95	20193	At2g28600	Putative ATP-dependent RNA helicase
2.08	3.57	13089	At2g27860	Putative dTDP-glucose 4-6-dehydratase
2.03	1.50	12312	At4g24780	Putative pectate lyase
2.02	1.43	15708	At2g34700	Putative proline-rich glycoprotein

Transcripts that show a constitutive (light-independent) twofold decrease in expression level in the *fhy3* or *far1* mutants relative to the wild-type values. The 'fold reduction' value is the mean transcript-signal value for dark-grown wild-type seedlings divided by the mean transcript-signal value for dark-grown mutant seedlings. See Supplementary Material for the full data set and criteria.

GGTTTGAACTCTTCCGACTTCTCAAAXACTTAAAATTTGGCAGTTAATTAXGTAGGTGTT. This is similar to the *Arabidopsis* MULE TIR fragment: GGAAAAAACCCCAAAAAATCCTCATTTAATTTTTATTTTTCCGTTTAATACCTACTTTAT. In *MuDR*, this includes the core transposase binding site (Benito and Walbot, 1997). As the *FLF* gene is a transcription factor, it may be affecting the other genes also found to be downregulated but which lack the binding motif. Other transcription factors, which are not represented on the microarray, also carry a similar sequence in regulatory regions (an example is the *GL1* gene, At3g27920) and so may be similarly regulated by FHY3.

Effect of mutations on light-regulated gene expression

We demonstrate in our microarray analysis that FAR1 and FHY3 are involved, whether directly or indirectly, in the transcriptional control of a number of genes. The *fhy3* mutant, in particular, shows measurably reduced responsiveness of the majority of light-regulated genes, implying that the protein has an early and general role in the light regulation of gene expression. However, none of the responses of early response genes defined by Tepperman *et al*. (2001) are strongly affected by these mutations. We infer from this that FAR1 and FHY3 do not affect the activity of these proposed primary response elements of the phyA transcriptional network. Light induction of a number of transcription factors is perturbed; but these are mostly affected irrespective of the light treatment. This may lead to the substantial differences observed in the expression profiles of a subset of downstream, less rapidly light-induced genes. The *far1* mutant shows a yet more subtle effect on the light regulation of gene expression, and its effects on light-regulated transcription are frequently outside the limits of the sensitivity of our assay to detect. In the case of a small number of strongly induced genes, such as the thionin in Figure 5(b), these effects are nonetheless clearly visible.

Effects on transcriptional cascade

It appears that the phenotypes of both *fhy3* and *far1* are a result of the combined effects of subtle changes in the responses of many different genes. These changes are likely to be, at least in part, a result of the altered expression of light-regulated transcription factors detailed in Figure 5(a). Although only the ZF1 protein shows a response difference of more than twofold, the combined effects of the mutant backgrounds on this important class of genes are likely to be substantial in their downstream effects. In addition, as less than 8000 of the approximately 25 000 *Arabidopsis* genes were assayed in our experiment, other transcription factor genes may be affected more strongly than those assayed and described here. The genes that are affected the most by the mutations, such as thionin and thioredoxin, may have binding sites for more than one of the strongly affected transcription factors in their promoters. The genes downstream of transcription factors that are induced normally in these mutants, such as the *CAB* genes known to be downstream of CCA1 (Wang and Tobin, 1998), mostly show less marked differences between wild-type and mutant responses (see Supplementary Material).

Correlation of phenotype with gene expression profiles

In neither of the mutants we examined, and in none of the genes assayed by our microarrays, are responses to light completely lost. The *phyA* mutant shows a complete loss of responsiveness to FRc, both in terms of gene expression and morphologic phenotype (Parks and Quail, 1993; Tepperman *et al*., 2001; Whitelam *et al*., 1993). The products of the *FAR1* or *FHY3* genes are, therefore, not necessary for FRc-responsive gene expression, as phyA is. The incomplete block of light signaling to gene expression in the mutants may be the result of multiple pathways leading to gene expression, of which that requiring FAR1 and FHY3 is only one (Figure 7). It has been proposed that this is because FAR1 and FHY3 perform degenerate, interchangeable molecular roles in the pathway leading from phyA to photomorphogenic development (Hudson *et al*., 1999; Wang and Deng, 2002). It is difficult to conclusively prove that this is the case without a set of multiple mutants in *FAR1, FHY3*, and their homologous genes, but we expect that a developmental phenotype as strong as that of *fhy3* is unlikely to be caused by a mutation in a single, highly degenerate pathway step. We propose that, rather than a partial block in a global pathway, *fhy3* has a severe loss of signaling activity in a branch pathway leading to gene expression concerned with cell expansion and morphologic development. The relatively severe morphologic phenotype of *fhy3*, in particular (Figure 1), contrasts with a restricted and subtle effect on light regulation of most genes, particularly on the early light-induced transcription factors (Figure 5). A strong effect of the *far1* and *fhy3* mutations is seen on a subset of genes that are likely to be involved in elongation growth, such as xyloglucan endotransglycosylases, which show strong deviation from wild-type responsiveness in the mutants (Figure 5b; Fry *et al*., 1992). The transcriptional control of this subset of downstream genes may be affected by the aberrant transcription-factor gene light responses in the mutants. This may explain the comparatively strong effect of loss of FAR1 or FHY3 function on the gross phenotype of the plant compared to the less obvious general effect on light-regulated gene expression. The control of cell extension growth in response to light may be a specific responsibility of the pathway of which the FAR1 and FHY3 proteins are a part. Such mutations would be expected to produce a photomorphogenical phenotype that is more obvious at the

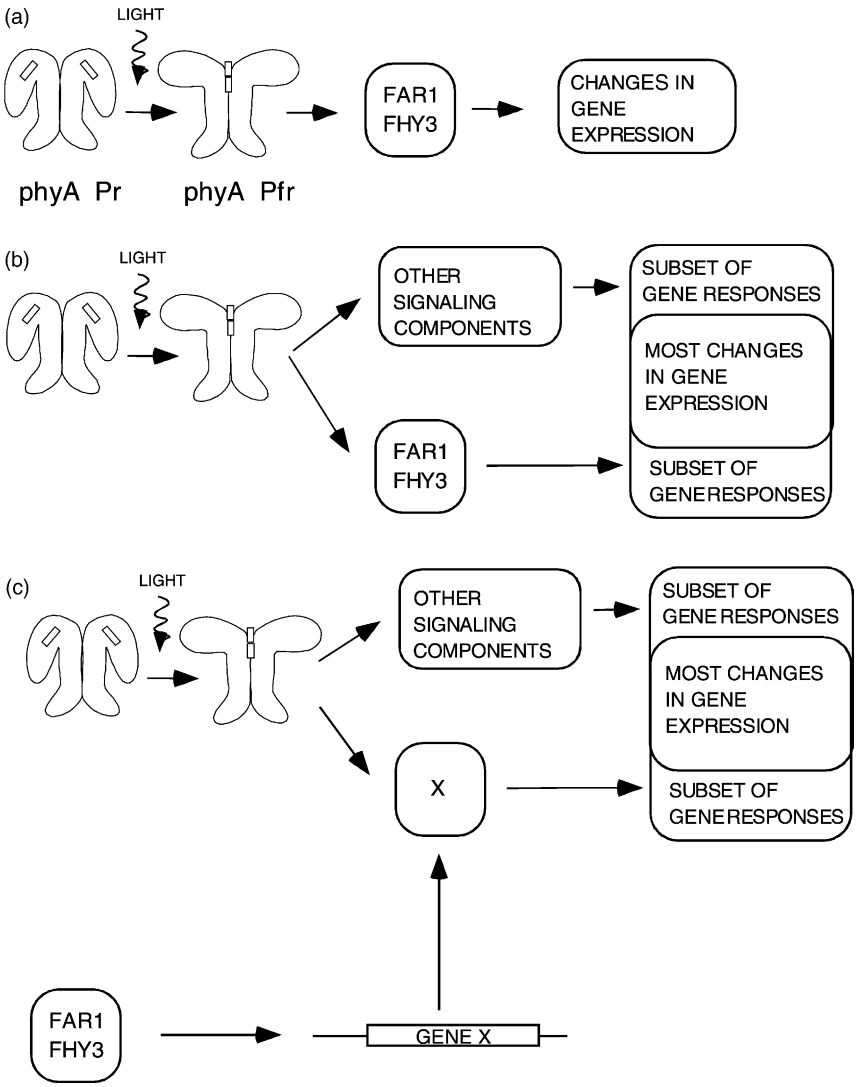

Figure 7. Models of the role of FAR1 and FHY3 in phytochrome signaling.
(a) The established view of FAR1 and FHY3, as a required module in a linear signaling pathway, where they regulate transcription of genes in direct response to light signals.
(b) FAR1 and FHY3 are required for the response of a subset of genes, and are capable of influencing the transcription of many others, as a part of a branched pathway from phytochrome. Some genes are regulated normally in the absence of these proteins.
(c) FAR1 and FHY3 lie outside the phytochrome-signal transduction pathway. Their absence creates a light-dependent phenotype because they are necessary for the expression of a light-signaling pathway component or components (X).

morphological level than at the level of light-induced gene expression. There is also a substantial effect on the light induction of an ACC oxidase gene that may contribute to the morphological effect of these mutations by an effect on ethylene biosynthesis.

The discontinuity between the severity of the morphological and molecular phenotypes of *fhy3* and *far1* is unexpected because the strength of the morphological response has been universally considered an indicator of the flux through the light-signaling pathway. The results presented here require a re-assessment of the connection between signaling and morphogenical phenotype. It is likely that the response of gene expression patterns to light is predominantly concerned with readying the seedling for photosynthesis. This is indicated by the predominance of chloroplast-related genes and cellular metabolic enzymes in the strong and sustained 'late' set of light-induced genes (Tepperman *et al.*, 2001). The predominant strategy of screening for long hypocotyls may have skewed the distribution of currently available mutants towards those that preferentially affect cell elongation growth. An important consequence of this is that the existing light-signal-transduction mutant collection may not represent the full signaling network regulating gene expression in response to light.

Light-independent transcriptional effects

The level of some transcripts is affected in the *far1* and *fhy3* mutants in a light-independent manner. This observation, together with that of Desnos *et al.* (2001) that *FHY1* expression is reduced in the *fhy3* background, defines a 'molecular phenotype' for etiolated seedlings of *far1* and *fhy3*. This molecular dark-grown phenotype is in direct contrast to the lack of a morphologic dark-grown phenotype; dark-grown seedlings of *far1* (Hudson *et al.*, 1999) and *fhy3*

(Whitelam *et al.*, 1993) are indistinguishable from the wild type. We therefore observe an effect on gene expression in etiolated seedlings that does not give rise to a visible phenotype without an external stimulus. This indicates that the skotomorphogenic developmental program probably does not require expression of these genes, but that attenuation of their expression results in faulty photomorphogenic development when the seedlings are exposed to light.

Interpretation of signaling mechanism

We consider that there are three plausible overall interpretations of the roles of FAR1 and FHY3 in the phyA-signaling pathway; the alternatives are illustrated in Figure 7. Our data (Figure 5a) show that *fhy3*, if not *far1*, slightly affects the transcription of some early induced transcription factor genes, such as *ZF1* and *MYB-106*, and thus that the primary role of *fhy3* may be upstream of any transcriptional cascade involved in photomorphogenesis. This would suggest a requirement for FAR1 and FHY3 in an upstream branch of the light-signaling pathway leading to light regulation of a subset of transcription factor genes, as illustrated in Figure 7(b). This proposal contrasts with the interpretation, suggested by the result of Wang and Deng (2002) that dominant-negative effects of FHY3 fragment expression all but shut down the phyA-signaling pathway, and that FAR1 and FHY3 are a required module of the phyA-signaling pathway (an interpretation illustrated in Figure 7a). After this paper was submitted, a study was published about the transcriptional light responses of several FR response mutants, using microarrays to examine only the phyA-regulated genes. The data was interpreted as evidence that the FAR1 and FHY3 proteins act upstream within the phyA-signaling pathway. However, while the data for the FHY3 fragment-expressing line were consistent with an interpretation such as that in Figure 7(a), the data for the *fhy3* and *far1* mutants were, as here, more consistent with a loss of function in a branch pathway as illustrated in Figure 7b (Wang *et al.*, 2002).

The observation that gene expression is also affected in dark-grown *far1* and *fhy3* raises the question whether or not the slight effects on early induced genes are a direct result of the mutations. The *far1* and *fhy3* proteins could have a dual role, both maintaining the expression of certain transcripts in darkness, and also participating in phytochrome signaling as pathway components. For example, the proteins could be phosphorylated, or they could bind to another protein, to modify their activity upon illumination. Alternatively, the effect on light-independent transcription could also cause under-expression of a protein or proteins required for light signaling. The cartoons in Figure 7(b,c) illustrate these possibilities. The combined effects of transcripts differently expressed in dark-grown seedlings may be enough to cause a photomorphogenic phenotype without direct involvement of FAR1 or FHY3 in light-regulated gene expression. We have direct evidence that this is at least partly the case from the *fhy1* mutant (Desnos *et al.*, 2001). Mutating the *FHY1* gene is sufficient to cause a loss of far-red light perception, and under-expression of this gene in *fhy3* could be sufficient to cause a significant photomorphogenic phenotype, comparable with that of the *fhy1* mutant itself. This possible mechanism is illustrated in Figure 7(c), with the gene X representing *FHY1*. We consider it unlikely that this is the sole link between FHY3 and photomorphogenesis because *far1* also shows a significant loss of far-red light perception (Hudson *et al.*, 1999) but does not show appreciably reduced *FHY1* transcript levels (Figure 6b). Nonetheless, this suggests a plausible mechanism by which transposase-related transcriptional activators such as FAR1 may affect photomorphogenesis. Several of the genes affected by the *far1* and *fhy3* mutations in the absence of light may be required for normal photomorphogenic development. The regulatory activity of FAR1 and FHY3 may be involved in creating the pre-conditioned gene-expression state, which, in turn, creates the proteome necessary for photomorphogenesis.

Experimental procedures

Sequence analysis and alignment

Sequence alignments were performed using MultAlin (Corpet, 1988). Affymetrix IDs were assigned to TAIR gene IDs by using a PERL script to find a perfect nucleotide match to the probe sequence. Where no exact match was found, a supervised BLAST search was used on the TAIR website (http://arabidopsis.org).

Yeast hybrid and in planta *transcriptional assays*

Yeast two-hybrid vectors from the Matchmaker 2.0 kit (Clontech, Palo Alto, CA, USA) were used to construct Gal4 activation and binding domain fusions of FAR1. Assays were performed in the Y187 strain as described (Ni *et al.*, 1998). *In planta* experiments used the enhancer trap system described by Moore *et al.* (1998), modified to carry proteins other than the Gal4 activation domain in the lacI fusion. Constructs carried hygromycin resistance (operator–reporter) and kanamycin (lacI fusion). Stably drug-resistant single-locus doubly homozygous lines in the Columbia-0 background were generated for assay by standard methods. GUS assays were performed by the fluorimetric method of Jefferson *et al.* (1987). The statistical test used to determine significance values in the GUS expression data was the one-tailed approximate *t*-test.

Plant growth and light sources

After pre-treatment as described by Hudson *et al.* (1999), induction of germination by a 3 h irradiation with white light, and subsequent storage for 21 h in the dark at room temperature, seeds for GUS analysis were transferred for 3 days to appropriate light conditions at 21°C. The light sources we used were described

elsewhere (Wagner *et al.*, 1991). Seed treatment and irradiation for the microarray experiments were exactly as described by Tepperman *et al.* (2001). The fluence rates of light were measured using a spectroradiometer (model LI-1800, Li-Cor, Lincoln, NE).

RNA preparation and Northern blotting

RNA was isolated from liquid nitrogen frozen seedlings by the method of Chang *et al.* (1993) and precipitated overnight at 4°C after the addition of 0.25 volumes of 10 M $LiCl_2$. A subsequent precipitation with 2.5 volumes of EtOH and 0.5 volumes of NH_4OAc was performed. Pellets were washed twice with 70% EtOH, vacuum-dried, and re-suspended in RNase-free water. Quality control by denaturing gel electrophoresis (A_{260}/A_{280} and A_{230}/A_{260} ratios) was used to ensure that all experimental samples were of sufficient integrity and purity. Northern blotting, probe labeling, phosphorimager detection, and 18 sec ratio calculations were performed according to the methods of Hoecker *et al.* (1999).

Microarray analysis of transcript expression

RNA extracted as above was converted to cDNA, labeled, denatured, hybridized to arrays, stained, and scanned according to the methods described by Tepperman *et al.* (2001), except that the procedures and equipment were in-house at Plant Gene Expression Center. Analysis of transcript expression was conducted mostly using Affymetrix Microarray Suite 5.0 (expression values given are 'signal' values) and Microsoft Excel together with custom macros. GENESPRING software (Silicon Genetics, Redwood City, CA, USA) was also used extensively. Probabilities and significance discussed in the text for expression data are derived from the two-tailed approximate Student's *t*-test.

Details are available at http://www.pgec.usda.gov/Quail/Hudson/

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Supplementary Material

An htm file with more information on this subject is available from the following website: http://www.blackwellpublishing.com/products/journals/suppmat/TPJ/TPJ1741/index.htm

References

Agarwal, A., Eastman, Q.M. and Schatz, D.G. (1998) Implications of transposition mediated by *V(D)J*-recombination proteins RAG1 and RAG2 for origins of antigen-specific immunity. *Nature,* **394**, 744–751.

Arabidopsis Genome Initiative (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature*, **408**, 796–815.

Ballesteros, M.L., Bolle, C., Lois, L.M., Moore, J.M., Vielle-Calzada, J.P., Grossniklaus, U. and Chua, N.H. (2001) LAF1, a MYB transcription activator for phytochrome A signaling. *Genes Dev.* **15**, 2613–2625.

Barkan, A. and Martienssen, R.A. (1991) Inactivation of maize transposon Mu suppresses a mutant phenotype by activating an outward-reading promoter near the end of Mu1. *Proc. Natl. Acad. Sci. USA*, **88**, 3502–3506.

Benito, M.I. and Walbot, V. (1997) Characterization of the maize Mutator transposable element MURA transposase as a DNA-binding protein. *Mol. Cell Biol.* **17**, 5165–5175.

Bolle, C., Koncz, C. and Chua, N.H. (2000) PAT1, a new member of the GRAS family, is involved in phytochrome A signal transduction. *Genes Dev.* **14**, 1269–1278.

Buche, C., Poppe, C., Schafer, E. and Kretsch, T. (2000) *eid1*: a new *Arabidopsis* mutant hypersensitive in phytochrome A-dependent high-irradiance responses. *Plant Cell*, **12**, 547–558.

Chang, S., Puryear, J. and Cairney, J. (1993) A simple and efficient method for isolating RNA from pine trees. *Plant Mol. Biol. Rep.* **11**, 113–116.

Corpet, F. (1988) Multiple sequence alignment with hierarchical clustering. *Nucl. Acids Res.* **16**, 10881–10890.

CSHL/WUGSC/PEB, Arabidopsis Sequencing Consortium (2000) The complete sequence of a heterochromatic island from a higher eukaryote. *Cell*, **100**, 377–386.

Desnos, T., Puente, P., Whitelam, G.C. and Harberd, N.P. (2001) FHY1: a phytochrome A-specific signal transducer. *Genes Dev.* **15**, 2980–2990.

Doak, T.G., Doerder, F.P., Jahn, C.L. and Herrick, G. (1994) A proposed superfamily of transposase genes: transposon-like elements in ciliated protozoa and a common 'D35E' motif. *Proc. Natl. Acad. Sci. USA*, **91**, 942–946.

Essers, L., Adolphs, R.H. and Kunze, R. (2000) A highly conserved domain of the maize activator transposase is involved in dimerization. *Plant Cell*, **12**, 211–224.

Fairchild, C., Schumaker, M.A. and Quail, P.H. (2000) HFR1 encodes an atypical bHLH protein that acts in phytochrome A signal transduction. *Genes Dev.* **14**, 2377–2391.

Fankhauser, C. and Chory, J. (1997) Light control of plant development. *Annu. Rev. Cell Dev. Biol.* **13**, 203–229.

Feschotte, C., Jiang, N. and Wessler, S.R. (2002) Plant transposable elements: where genetics meets genomics. *Nat. Rev. Genet.* **3**, 329–341.

Fry, S.C., Smith, R.C., Renwick, K.F., Martin, D.J., Hodge, S.K. and Matthews, K.J. (1992) Xyloglucan endotransglycosylase, a new wall-loosening enzyme activity from plants. *Biochem. J.* **282**, 821–828.

Haren, L., Ton-Hoang, B. and Chandler, M. (1999) Integrating DNA: transposases and retroviral integrases. *Annu. Rev. Microbiol.* **53**, 245–281.

Hisada, A., Hanzawa, H., Weller, J.L., Nagatani, A., Reid, J.B. and Furuya, M. (2000) Light-induced nuclear translocation of endogenous pea phytochrome A visualized by immunocytochemical procedures. *Plant Cell*, **12**, 1063–1078.

Hoecker, U., Tepperman, J.M. and Quail, P.H. (1999) SPA1, a WD-repeat protein specific to phytochrome A signal transduction. *Science*, **284**, 496–499.

Hsieh, H.L., Okamoto, H., Wang, M., Ang, L.H., Matsui, M., Goodman, H. and Deng, X.-W. (2000) FIN219, an auxin-regulated gene, defines a link between phytochrome A and the downstream regulator COP1 in light control of *Arabidopsis* development. *Genes Dev.* **14**, 1958–1970.

Hudson, M.E. (2000) The genetics of phytochrome signalling in *Arabidopsis*. *Semin. Cell Dev. Biol.* **11**, 475–483.

Hudson, M., Ringli, C., Boylan, M.T. and Quail, P.H. (1999) The FAR1 locus encodes a novel nuclear protein specific to phytochrome A signaling. *Genes Dev.* **13**, 2017–2027.

Jefferson, R.A., Kavanagh, T.A. and Bevan, M.W. (1987) GUS fusions: beta-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. *EMBO J.* **6**, 3901–3907.

Kidwell, M. and Lisch, D. (1997) Transposable elements as sources of variation in animals and plants. *Proc. Natl. Acad. Sci. USA*, **94**, 7704–7711.

Kircher, S., Kozma-Bognar, L., Kim, L., Adam, E., Harter, K., Schafer, E. and Nagy, F. (1999) Light quality-dependent nuclear import of the plant photoreceptors phytochrome A and B. *Plant Cell*, **11**, 1445–1456.

Lander, E.S., Linton, L.M., Birren, B. *et al.* (2001) Initial sequencing and analysis of the human genome. *Nature*, **409**, 860–921.

Lisch, D.R. (2002) Mutator transposons. *Trends Plant Sci.* **7**, 498–504.

Lisch, D.R., Freeling, M., Langham, R.J. and Choy, M.Y. (2001) Mutator transposase is widespread in the grasses. *Plant Physiol.* **125**, 1293–1303.

Martienssen, R.A., Barkan, A., Freeling, M. and Taylor, W.C. (1989) Molecular cloning of a maize gene involved in photosynthetic membrane organization that is regulated by Robertson's Mutator. *EMBO J.* **8**, 1633–1639.

Martinez-Garcia, J.F., Huq, E. and Quail, P.H. (2000) Direct targeting of light signals to a promoter element-bound transcription factor. *Science*, **288**, 859–863.

Moller, S.G., Kunkel, T. and Chua, N.H. (2001) A plastidic ABC protein involved in intercompartmental communication of light signaling. *Genes Dev.* **15**, 90–103.

Moore, I., Galweiler, L., Grosskopf, D., Schell, J. and Palme, K. (1998) A transcription activation system for regulated gene expression in transgenic plants. *Proc. Natl. Acad. Sci. USA*, **95**, 376–381.

Ni, M., Tepperman, J.M. and Quail, P.H. (1998) PIF3, a phytochrome-interacting factor necessary for normal photoinduced signal transduction, is a novel basic helix-loop-helix protein. *Cell*, **95**, 657–667.

Ni, M., Tepperman, J.M. and Quail, P.H. (1999) Binding of phytochrome B to its nuclear signalling partner PIF3 is reversibly induced by light. *Nature (London)*, **400**, 781–784.

Parks, B.M. and Quail, P.H. (1993) *hy8*, A new class of *Arabidopsis* long hypocotyl mutants deficient in functional phytochrome A. *Plant Cell*, **5**, 39–48.

Quail, P.H. (1998) The phytochrome family: dissection of functional roles and signalling pathways among family members. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **353**, 1399–1403.

Raizada, M.N., Benito, M.I. and Walbot, V. (2001a) The MuDR transposon terminal inverted repeat contains a complex plant promoter directing distinct somatic and germinal programs. *Plant J.* **25**, 79–91.

Raizada, M.N., Brewer, K.V. and Walbot, V. (2001b) A maize MuDR transposon promoter shows limited autoregulation. *Mol. Genet. Genomics*, **265**, 82–94.

Singer, T., Yordan, C. and Martienssen, R.A. (2001) Robertson's *Mutator* transposons in *A. thaliana* are regulated by the chromatin-remodeling gene *Decrease DNA Methylation (DDM1)*. *Genes Dev.* **15**, 591–602.

Soh, M.S., Hong, S.H., Hanzawa, H., Furuya, M. and Nam, H.G. (1998) Genetic identification of FIN2, a far red light-specific signaling component of *Arabidopsis thaliana*. *Plant J.* **16**, 411–419.

Tepperman, J.M., Zhu, T., Chang, H.S., Wang, X. and Quail, P.H. (2001) Multiple transcription-factor genes are early targets of phytochrome A signaling. *Proc. Natl. Acad. Sci. USA*, **98**, 9437–9442.

Wagner, D., Tepperman, J.M. and Quail, P.H. (1991) Overexpression of phytochrome B induces a short hypocotyl phenotype in transgenic *Arabidopsis*. *Plant Cell*, **3**, 1275–1288.

Walbot, V., Britt, A.B., Luehrsen, K., Mclaughlin, M. and Warren, C. (1988) Regulation of mutator activities in maize. *Basic Life Sci.* **47**, 121–135.

Wang, H. and Deng, X.W. (2002) *Arabidopsis* FHY3 defines a key phytochrome A signaling component directly interacting with its homologous partner FAR1. *EMBO J.* **21**, 1339–1349.

Wang, H., Ma, L., Habashi, J., Li, J., Zhao, H. and Deng, X.-W. (2002) Analysis of far-red light regulated genome expression profiles of phytochrome A pathway mutants in *Arabidopsis*. *Plant J.* **32**, 723–733.

Wang, Z.Y. and Tobin, E.M. (1998) Constitutive expression of the *CIRCADIAN CLOCK ASSOCIATED 1 (CCA1)* gene disrupts circadian rhythms and suppresses its own expression. *Cell*, **93**, 1207–1217.

Whitelam, G.C., Johnson, E., Peng, J., Carol, P., Anderson, M.L., Cowl, J.S. and Harberd, N.P. (1993) Phytochrome A null mutants of *Arabidopsis* display a wild-type phenotype in white light. *Plant Cell*, **5**, 757–768.

Xu, W., Purugganan, M.M., Polisensky, D.H., Antosiewicz, D.M., Fry, S.C. and Braam, J. (1995) *Arabidopsis* TCH4, regulated by hormones and the environment, encodes a xyloglucan endotransglycosylase. *Plant Cell*, **7**, 1555–1567.

Yanovsky, M.J., Casal, J.J. and Luppi, J.P. (1997) The VLF loci, polymorphic between ecotypes Landsberg erecta and Columbia, dissect two branches of phytochrome A signal transduction that correspond to very low-fluence and high-irradiance responses. *Plant J.* **12**, 659–667.

Zeidler, M., Bolle, C. and Chua, N.H. (2001) The phytochrome A specific signaling component PAT3 is a positive regulator of *Arabidopsis* photomorphogenesis. *Plant Cell Physiol.* **42**, 1193–1200.